REMARKS

With this response, claims 46-97 are pending. Claims 1-45 have been canceled. Support for claim 46 can be found in the specification at p. 10, lines 14-28; p.12, lines 3-4; p. 18, lines 31-32; and original claim 1. Support for claims 47, 48, 49, 67, 68, and 69 can be found in the specification at p. 12, lines 3-5 and lines 7-10; and original claims 2 and 3. Support for claims 50, 51, 74, 75, 90, and 91 can be found in the specification at p. 16, lines 20-23. Support for claims 52, 53, 76, 77, 92, and 93 can be found in the specification at p. 11, lines 29-31. Support for claims 54, 55, 56, 78, 79, 80, 94, and 95 can be found in the specification at p. 19, lines 10-14. Support for claims 57, 58, 81, and 82 can be found in the specification at p. 18, line 33 through p. 19, line 1. Support for claims 59, 60, 83, 84, 96, and 97 can be found in the specification at p. 18, lines 23-26. Support for claims 61, 62, 63, 85, 86, and 87 can be found in the specification at p. 4, lines 1-13 and p. 10, lines 5 and 6. Support for claim 64 can be found in the specification at p. 10, lines 14-16 and lines 24-26; p. 12, lines 3-4; p. 18, lines 31-32; and original claim 10. Support for claim 65 can be found in the specification at p. 10, lines 24-26. Support for claim 66 can be found in the specification at p. 11, lies 8-10. Support of claims 70, 71, 72, and 73 can be found in the specification at p. 16, line 32 through p. 17, line 30, and original claims 13 and 14. Support for claims 88 and 89 can be found in the specification at p. 14, lines 8-22, and original claims 34 and 35.

I. 35 U.S.C. 102(b)

Applicants acknowledge the Office's reconsideration and withdrawal of the rejection of claims 1-3 and 17 as being unpatentable over Haugland et al. (U.S. Patent No. 5,436,134) and Yue (U.S. Patent No. 5,656,449) and claims 1, 2, 4, 10, 11, 13, and 17 as being unpatentable over Fitzpatrick-McElligott (U.S. Patent No. 5,466,587).

II. 35 U.S.C. 103(a)

A. Haugland et al. and Yue in view of Magrassi et al., Gan et al., and Gee et al., and further in view of Pichersky and Sanford et al.

Reconsideration is requested of the rejection of claims 1-19 and 41-44 under 35 U.S.C. 103(a) as being unpatentable over Haugland et al. (U.S. Patent No. 5,436,134) and Yue (U.S. Patent No. 5,656,449) in view of Magrassi et al. (1987), Gan et al. (1999) and Gee et al. (U.S. Patent No. 5,888,829), and further in view of Pichersky (U.S. Patent No. 5,436,134) and Sanford et al. (U.S. 4,945,050).

Claims 1-19 and 41-44 have been canceled, thus rendering the rejection moot as applied thereto. These claims, however, have been replaced by claims 46-87 which are patentable over the cited references for the following reasons.

Claim 46 is directed to a method for individually labeling a cell within a population of cells. The method comprises propelling a particle coated with a lipophilic hydrophobic dye at the population of cells to cause the particle to contact the membrane of the cell, and allowing the dye to diffuse into the

cell membrane and thereby differentially label the cell relative to neighboring cells within the population.

Haugland et al. disclose novel dyes. These dyes "have utility in any current application for detection of nucleic acids that requires a sensitive detection agent."[1] The dyes are disclosed to be useful for the "detection of nucleic acids in a variety of cells and tissues."[2] It is further disclosed that while "many of the dyes have shown an ability to **permeate cellular membranes rapidly and completely** . . . any other technique that is suitable for transporting the dye **across cell membranes** with minimal disruption of the viability of the cell and integrity of cell membranes is also a valid method of combining the sample with the subject dye."[3] Examples of such suitable methods of delivery are disclosed to include "bombardment with solid particles coated with or in the presence of the dyes,"[4] although none of the examples demonstrate the use of bombardment. As the dyes disclosed in Haugland et al. are for the staining of nucleic acids, the methods of Haugland et al., therefore, would necessitate that the dyes **pass through or be transported across cell membranes**, for example, the cell or nuclear membrane, in order to stain nucleic acids contained within the cell. Accordingly, Haugland et al. do not disclose labeling cells by staining cell membranes or labeling cells in

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[1] Haugland et al., column 2, lines 17-19.

[2] Haugland et al., column 2, lines 23-24.

[3] Haugland et al., column 7, lines 47-53 (emphasis added).

[4] Haugland et al., column 7, lines 59-61.

the absence of dye permeating or being transported across cellular membranes.

Yue discloses novel dyes and the use of those dyes to stain a sample containing a nucleic acid, incubating the sample for a time sufficient to obtain a detectable fluorescent response, and observing the fluorescent response. While Yue states that suitable means for transporting the dyes **across cell membranes** and into biological structures, such as cells, includes "bombardment with solid particles coated with or in the presence of the dyes,"[5] none of the examples demonstrate the use of bombardment. As the dyes disclosed in Yue are for the staining of nucleic acids, the methods of Yue, therefore, would necessitate that the dyes **pass through or be transported across cell membranes**, for example, the cell or nuclear membrane, in order to stain nucleic acids contained within the cell. Accordingly, Yue does not disclose labeling cells by staining cell membranes or labeling cells in the absence of dye permeating or being transported across cellular membranes.

Magrassi et al. disclose the staining of living motor nerve terminals by immersion of rat muscles into a staining solution containing one of eighteen dyes disclosed therein, thereby staining **all** motor nerve terminals contained within the immersed muscles. Magrassi et al. do not disclose labeling individual cells or labeling individual cells by propelling dye-coated particles into contact with cell membranes.

Gan et al. disclose labeling individual axon terminals by iontophoretic application of lipophilic dyes such as DiO and DiI.

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[5] Yue, column 9, lines 64-65.

Gan does not disclose labeling individual cells by propelling dye-coated particles into contact with cell membranes.

Gee et al. disclose the use of caged ionophores - molecules that act as ion-transport agents that have been caged by a chemical moiety that when bound to the ionophore reduces or prevents the biological activity of the same.[6] One particular ionophore used in Gee et al. is the Ca^{2+} ion indicator Oregon Green® BAPTA. Gee et al. do not disclose labeling cells by propulsion of dye-coated particles, labeling cells by staining cell membranes, or labeling cells using lipophilic hydrophobic dyes.

Pichersky discloses the delivery of DNA coated particles **into cells** by acceleration, such as by the Biolistics® Particle Delivery System. Pichersky further states that the particles can be accelerated through a stainless steel or Nytex screen. It is believed that the screen reduces the size of aggregate particles and leads to a higher frequency of transformation by reducing damage inflicted on the recipient cells by projectiles that are too large. Because Pichersky discloses delivering DNA coated particles, the method of delivery necessitates delivery of the particles **into the nucleus** of the cell. Pichersky does not disclose labeling cells by propulsion of dye-coated particles, labeling cells by staining cell membranes, or labeling cells using lipophilic hydrophobic dyes.

Sanford et al. disclose methods of introducing particles into the interior of cells, wherein they become incorporated into

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[6] Gee et al., column 2, lines 41-48.

the same.[7] The particles may be coated with biological substances, including fluorescent probes. The emphasis of the methods disclosed therein is upon entry of the particle into the interior of the cell. Specifically, according to the method disclosed by Sanford et al., "particles of the appropriate size, accelerated to appropriate velocities can readily penetrate thin barriers such as cell walls and cell membranes, thereby entering into the cell cytoplasm"[8] such that "[o]nce in the aqueous environment of the cytoplasm, the biological substances would **dissolve to be dispersed in the cyto-solution.**"[9] Hence, the delivery of any substance contained on the particle into the cell is conditioned upon the particle's entry into the cytoplasm and the dissolving and dispersion of the substance into the cyto-solution. Sanford et al. do not disclose labeling cells by propulsion of dye-coated particles, labeling cells by staining cell membranes, or labeling cells using lipophilic hydrophobic dyes.

The Office has failed to establish a *prima facie* case of obviousness, as the references, when combined, fail to teach or suggest each and every limitation of claim 46.[10] Specifically, when combined the references fail to teach or suggest methods for labeling a cell wherein a lipophilic hydrophobic dye is used to label the **cell membrane** by propelling a coated particle at the cell thereby causing the particle to contact the membrane of the

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[7] Sanford et al., claim 1, column 13, lines 38-44.

[8] Sanford et al., column 5, lines 30-33.

[9] Sanford et al., column 6, lines 62-64 (emphasis added).

[10] MPEP §2142.

cell, and allowing the dye to diffuse into the cell membrane and label the cell. As transport of the dye (or biological substance) contained on the particles **across cell membranes** is **taught and required** by the primary references Haugland et al. and Yue, and by the secondary references Sanford et al. and Pichersky, any combination of these references alone or additionally in combination with Magrassi et al., Gan et al., and Gee et al., fails to teach or suggest each and every claim limitation. Accordingly, the Office has failed to establish a *prima facie* case of obviousness.[11]

In addition, the Office has failed to establish a *prima facie* case of obviousness as there is no motivation or suggestion within the references themselves to modify or combine the reference teachings.[12] Haugland et al., Yue, Sanford et al., and Pichersky, each of which discloses particle bombardment, specifically disclose propelling particles and the dyes or biological substances contained thereon **across cell membranes** in order to deliver the dyes into the **solutions of the cells** (e.g., the cytoplasm or the nucleoplasm). None of these references suggests the use of particle bombardment to contact cell membranes and label the same by diffusion of the dye into the cell membrane. Indeed, Haugland et al., Yue, Pichersky, and Sanford et al. would teach away from the presently claimed invention, disclosing that the particles and substances contained thereon must enter into the cytoplasm or nucleoplasm of a cell in order for the cell to be stained. Accordingly, one of skill in

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[11] MPEP §2142.

[12] MPEP §2142.

the art would not be motivated to combine these references with Magrassi et al., Gan et al., and Gee et al., which discuss particular dyes and cell labeling by means other than particle bombardment. Because there is no motivation or suggestion within the references themselves to modify or combine the reference teachings, the Office has failed to establish a *prima facie* case of obviousness.[13]

Claims 47-63 depend from claim 46 and are patentable over Haugland et al. and Yue in view of Magrassi et al., Gan et al., and Gee et al., and further in view of Pichersky and Sanford et al. for the reasons stated above with respect to claim 46 and by reason of the additional requirements which they introduce.

Claim 64 is directed to a method for individually labeling cells of a population of cells. The method comprises propelling a plurality of particles coated with a lipophilic hydrophobic dye at the population of cells to cause the particles to contact the membranes of the cells, and allowing the dye to diffuse into the cell membranes and thereby differentially label the cells relative to neighboring cells within the population. Haugland et al., Yue, Magrassi et al., Gan et al., Gee et al., Pichersky, and Sanford et al., do not individually or in combination, suggest labeling cells by propelling a plurality of lipophilic hydrophobic dye-coated particles into contact with the membranes of the cells, and allowing the dyes to diffuse into the cell membranes thereby differentially labeling the cells relative to neighboring cells within the population. If anything, Haugland et al., Yue, Pichersky, and Sanford et al. would teach away from the presently claimed invention, teaching that the particles and

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[13] MPEP §2142.

substances contained thereon must enter into the cytoplasm or nucleoplasm of a cell in order for the cell to be stained.

Claims 65-87 depend from claim 64 and are patentable over Haugland et al. and Yue in view of Magrassi et al., Gan et al., and Gee et al., and further in view of Pichersky and Sanford et al. for the reasons stated above with respect to claim 64 and by reason of the additional requirements which they introduce.

Claims 88-97, discussed in further detail below, are generally directed to methods for individually labeling cells within a population of cells whereby the cells are differentially labeled relative to neighboring cells within the population, the methods comprising propelling a plurality of particles containing a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra at the population of cells to cause the particles to enter the cells, and allowing expression of the proteins encoded by the nucleotide sequences to occur and thereby differentially label the cells relative to neighboring cells within the population. Haugland et al., Yue, Magrassi et al., Gan et al., Gee et al., Pichersky, and Sanford et al., do not suggest, individually or in combination, labeling cells by propelling a plurality of particles containing a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra at the cells. Accordingly, claims 88-97 are patentable over these references.

B. Wong et al in view of Tsien and Matz et al. and further in view of Sanford et al.

Reconsideration is requested of the rejection of claims 34-38 and claim 45 under 35 U.S.C. 103(a) as being unpatentable over

Wong et al. (1998; ref 32) in view of Tsien (1998) and Matz et al. (1999) and further in view of Sanford et al. (U.S. 4,945,050). Although the Office rejected claims 34-38, claims 37 and 38 were withdrawn from consideration by the Office as being drawn to a non-elected group. Applicants assume the Office intended instead to reject claims 34-36 and will treat the rejection as such.

Claims 34-38 and 45 have been canceled, thus rendering the rejection moot as applied thereto. These claims, however, have been replaced by claims 82-91 which are patentable over the cited references for the following reasons.

Claim 88 is directed to a method for individually labeling cells within a population of cells whereby the cells are differentially labeled relative to neighboring cells within the population. The method comprises propelling a plurality of particles containing a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra at the population of cells to cause the particles to enter the cells, and allowing expression of the proteins encoded by the nucleotide sequences to occur and thereby differentially label the cells relative to neighboring cells within the population.

Wong et al. discloses staining retinal ganglion cells with green fluorescent protein ("GFP") by biolistic methods. Wong et al. do not disclose use of a plurality of particles or the use of a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra.

Tsien discloses that there are many different GFPs having different fluorescent properties. Although it is asserted that Tsien discloses that different proteins are useful as multiple labels and reporters, such disclosure was made in reference to

future research into class 4 GFP mutants and how the same could be used in fluorescence resonance energy transfer (FRET), a technique that is conceptually different and unrelated to the presently claimed method. Tsien does not disclose labeling cells by propulsion of particles labeled with nucleotide sequences, use of a plurality of particles, or use of a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra.

Matz et al. discloses that there are many fluorescent proteins that are analogous to GFP and that can be used for the same purpose. Matz et al. do not disclose labeling cells by propulsion of particles labeled with nucleotide sequences, use of a plurality of particles, or use of a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra.

Sanford et al. disclose methods of introducing particles into the interior of cells, wherein they become incorporated into the interior of the cells.[14] The particles may be coated with biological substances, including nucleic acids such as DNA and RNA.

The Office has failed to establish a *prima facie* case of obviousness, as the references, when combined, fail to teach or suggest each and every limitation of claim 82.[15] Specifically, each of these references fails to teach or suggest a method of individually labeling cells utilizing a plurality of nucleotide sequences encoding fluorescent proteins having different emission

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[14] Sanford et al., claim 1, column 13, lines 38-44.

[15] MPEP §2142.

spectra. While Tsien and Matz et al. disclose multiple GFPs having different fluorescent properties, Wong et al. disclose labeling a cell by biolistic transfection with GFP, and Sanford et al. disclose the biolistic introduction of particles that may contain DNA or RNA, none of these references, alone or in combination, teaches or suggests individually labeling cells by propelling at the cells a plurality of particles containing a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra to label the cells. Indeed, there is nothing within the references that would teach or suggest labeling cells in a manner other than by using nucleotide sequences **encoding a single GFP** or a protein analogous to GFP. Labeling of cells by use of a plurality of nucleotide sequences encoding fluorescent proteins having different emission spectra, versus use of nucleotide sequences encoding a single fluorescent protein, allows for differential labeling of cells such that one can differentiate not only labeled from non-labeled cells, but one can also differentiate among adjacent labeled cells. In the absence of the use of a plurality of nucleotide sequences as required by claim 88 differential labeling cannot be achieved. Because the references fail to teach or suggest each and every claim limitation, the Office has failed to establish a *prima facie* case of obviousness.[16]

In addition, the Office has failed to establish a *prima facie* case of obviousness as there is no motivation or suggestion within the references themselves to modify or combine the

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[16] MPEP §2142.

reference teachings.[17] While Tsien and Matz et al. disclose multiple GFPs having different fluorescent properties and Wong et al. disclose labeling a cell by biolistic transfection with a single GFP, there is nothing within any of the above-mentioned references that would suggest labeling a cell by using more than a single GFP or GFP analog. Therefore, there is no motivation or suggestion within the references to combine or modify the same to achieve the claimed method. Sanford et al. do nothing to cure this defect, as they merely disclose the biolistic introduction of particles that may contain DNA or RNA into cells. Because there is no motivation or suggestion within the references themselves to modify or combine the reference teachings, the Office has failed to establish a *prima facie* case of obviousness.[18]

Claims 89-97 depend from claim 88 and are patentable over Wong et al. in view of Tsien and Matz et al. and further in view of Sanford et al. for the reasons stated above with respect to claim 88 and by reason of the additional requirements which they introduce.

Claims 46-87 are discussed above. Wong et al., Tsien, Matz et al., and Sanford et al. do not individually or in combination, suggest labeling cells by propelling lipophilic hydrophobic dye-coated particles into contact with the cell membranes. Accordingly, claims 46-87 are patentable over these references.

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[17] MPEP §2142.

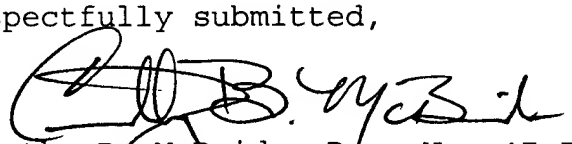
[18] MPEP §2142.

CONCLUSION

In light of the above arguments, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. 103(a).

Applicants request an extension of time to and including October 7, 2003, for filing a response to the above-mentioned Office action.

Respectfully submitted,



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